ON THE FORMATION OF S-(α,β-DICARBOXYETHYL) DERIVATIVES OF GLUTAMIC-ASPARTIC AMINOTRANSFERASE

C. Turano, A. Giartosio, F. Riva and P. Fasella

Institute of Biological Chemistry, the University of Rome and Centro di Enzimologia del C.N.R., Rome, Italy

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The content and function of thiol groups in pure preparations of aspartic aminotransferase from pig heart have been studied in several laboratories (Polyanovsky et al., I963; Turano et al., I963). While all authors agree that the blocking of SH groups results in inhibition of the enzyme activity, a disagreement exists over the number of SH groups present per mole of enzyme. Turano et al. (I963) found 6-7 SH groups per mole (assuming a molecular weight of 58,000), while Polyanovsky et al. (I963) found considerably less. Since the preparations used by the two groups of investigators were obtained by different procedures, it seemed reasonable to assume that the discrepancy was the consequence of modifications undergone by one or both preparations in the course of purification. A survey of the purification procedures shows that a prominent feature of one of them is heating in the presence of maleate (Jenkins et al., I959). Since maleate is known to form stable derivatives with -SH groups (Morgan et al., I938; Calan et al., I963), it was suspected that some of the thiol groups of the enzyme prepared by the heat treatment had reacted with maleate to give S-(α,β-dicarboxyethyl) derivatives and for this reason were not available to SH reagents. To test this hypothesis the enzyme was prepared in the four different ways des-

cribed below: a) according to Jenkins et al. (I959) with modifications suggested by Polyanovsky et al. (I963); b) as described in a) except that succinate was used instead of maleate; c) according to Lis (1958) with minor modifications; d) according to Lis (1958) and then treated with maleate as follows: 3 ml of 9 x IO^{-5} M enzyme were added with 3 ml of 0.1 M sodium maleate buffer, pH 6, containing 0.01 M EDTA and with 0.3 ml of 50 mM sodium ketoglutarate; the reaction mixture was heated at 75°C for 20 minutes, cooled and dialyzed for 40 hrs. against 4 changes of distilled water (five liters each).

Samples of the four above enzyme preparations were analyzed for specific activity (Lis, 1958; Turano et al. 1963), SH groups (Boyer, 1954) and amino acid composition (Moore et al., 1958).

The results concerning the specific activity and the SH groups titrations are reported in Table I. As shown in the table, the specific activity does not vary greatly in the various preparations, while the number of titratable SH groups drops by approximately one per mole in the two enzyme preparations which have been treated with maleate.

For the determination of the amino acid composition, samples of the four enzyme preparations were hydrolyzed in 6 N HCl (at 110°C for 24 hrs) and analyzed by bidimensional paper chromatography, paper electrophoresis (see Table 2) and by quantitative column chromatography (Moore et al., I958). The analyses showed that the hydrolyzates of enzyme preparations that have been exposed to maleate contained, beside the amino acids known to be present in aspartic aminotransferase from pig heart (Turano et al., 1963), an unknown ninhydrin positive spot which, in electrophoresis, migrated to the anode considerably faster than aspartic acid. These acidic properties and the coincidence between its appearance and the loss of SH groups suggested that the unknown substance

TABLE 1

COMPARISON OF SOME PROPERTIES OF GLUTAMIC ASPARTIC AMINOTRANSFERASE PREPARED FROM PIG HEART BY DIFFERENT METHODS

Method of preparation and treatment	Specific activity (Lis, 1958) units/mg of protein	SH groups titrable in 8 M urea (Boyer, 1954)	Equivalents of S-(α,β-dicarboxyethyl)cysteine the acid hydrolyzate
Lis (1958)	7,200	6.3	none
Lis (1958) after treatment with maleate	6,500	4.9	1.0
Jenkins et al.(1959) modified by Polyanovsky (1963)	6,400	4.8	1.1
As above except that succinate was used instead of maleate	7,400	5.9	none

TABLE 2

COMPARISON OF SOME PROPERTIES OF THE UNKNOWN SUBSTANCE ISOLATED FROM THE ACID HYDROLYZATES OF MALEATE-TREATED ASPARTIC AMINOTRANSFERASE AND OF SYNTHETIC S-(α,β-DICARBOXYETHYL)CYSTEINE

Paper chromatography(°)		
Rf values in:		
n-butanol/acetic acid/water (4:I:5)	0.32	0.32
phenol saturated with water	0.11	0.11
Column chromatography(°°)		
elution volume in ml	102-107	102-107
Paper electrophoresis		
mobility in cm/hr(°°°)	7.8	7.9
Specific reactions		
ninhydrin	+	+
cyanide-Na nitroprusside test for SS and SH groups (Toennies, I95I)	-	-
Na azide test for divalent sulphur (Sjöquist,I953)	+	+
Platinic iodide test for divalent sulphur (Toennies, I95l)	+	+

(°) descending on n. 4 Whatman paper;
(°°) from a 0.9xI50 cm column of Amberlite IR I20 eluted with pH 3.25, 0.2 M sodium citrate buffer, according to Moore et al. (1958)
(°°°) on 3 mm Whatman paper, using a pH 3.6 pyridine/acetic acid/water buffer, with a voltage gradient of 30 volt/cm.

could be identified with the stable thioether which maleate is known to form with cysteine, i.e., S(α,β-dicarboxyethyl)cysteine. To test this hypothesis, the latter compound was synthetized (Calan et al., I963) and its properties compared with those of the unknown substance. The res-

ults are reported in Table 2. The addition of the synthetic compound to the maleate treated enzymes either before or after the hydrolysis did not cause the appearance of any new spot on the chromatograms or electrograms, but only intensified the one corresponding to the unknown substance. Addition of the synthetic compound to the enzyme prepared according to Lis (1958) before the hydrolysis caused the appearance of the same spot.

A quantitative analysis of the hydrolyzate, carried out by the method of Moore et al. (1958) calibrated against samples of the synthetic compound, showed that both of the preparations which had been treated with maleate contained one equivalent of S-(α,β -dicarboxyethyl)cysteine per mole of enzyme, assuming a molecular weight of 58,000.

These results account quantitatively for the difference between the number of SH groups titratable in the enzyme prepared with or without maleate treatment, and might also explain some differences between their isoelectric properties.

Concerning the relations between structure and function, the specific activity, as assayed under standard conditions, is not greatly affected by the blocking of one SH group by maleate; the change in activity is, in fact, comparable to that obtained by the addition of one equivalent of p-mercuribenzoate (Turano et al., 1963).

Of the several procedures for the purification of holoaspartic aminotransferase from pig heart, the one proposed by Jenkins et al. (1959) offers the greatest advantages in terms of rapidity, yield and applicability to large amounts of material. When following this procedure, it may be convenient, considering the present results, to substitute maleate with succinate.

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